44. (New) The modified nucleic acid of claim 3, wherein the mammalian cell is a mammary epithelial cell.

45. (New) The modified nucleic acid of claim 4, wherein the mammalian cell is a mammary epithelial cell.

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46. (New) The modified nucleic acid of claim 23, wherein the mammalian cell is a mammary epithelial cell.

47. (New) The method of claim 10, wherein the mammalian cell is a mammary epithelial cell.

REMARKS

Claims 1-11, 17-21 and 23-47 are pending. Claims 1-11, 17 and 19-26 have been amended. Claims 12-16 and 22 have been cancelled without prejudice. However, the amendments to and/or the cancellation of the claims were made solely to expedite prosecution of the present application. New claims 27-47 were added. Support for the new claims can be found throughout the application as originally filed. No new matter has been added.

Rejection of Claims 1-26 Under 35 U.S.C. §112, second paragraph

Claims 1-26 are rejected under 35 U.S.C. §112, second paragraph "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention."

In particular, the Examiner states that claims 1-26 are indefinite "because the recite 'the gene', or 'the known gene', or 'the natural gene' without antecedent basis." Claims 12-16 and 22 have been cancelled without prejudice, thereby obviating the Examiner's rejection to these claims. The remaining claims have been amended to overcome this rejection.

The Examiner further rejected claims 1-12, 22, 24 and 25 as being indefinite "because they recite a 'known nucleic acid' the specification does not define what is encompassed by the

genus of 'known' nucleic acids." The term "known" has been removed from the claims, thereby obviating this rejection.

Claims 2, 7, 10 and 11 are also rejected as being

> indefinite because they recite 'the replaced codon' without antecedent basis. These claims were drawn to the replacement of an mRNA instability motif with a codon. The specification only teaches one instability motif, and it consists of 5 nucleotides. It is noted that replacement of 5 nucleotides with a codon will result in a frameshift.

Claims 2, 7, 10 and 11 have been amended to recite that "at least a portion of the mRNA instability motif" is replaced by a preferred codon. The amendment to these claims obviates the Examiner's rejection.

Claims 2, 3, and 7 have also been amended to recite a "nucleic acid encoding a parasite protein" to obviate the Examiner's rejection of these claims.

With regards to claims 3-12, 17-21 and 26, the Examiner states that they "are indefinite because they recite 'a milk protein-specific codon' or 'a mammary-specific codon'." In particular, the Examiner states that "these terms are not defined in the specification . . . [and] in general, the terms milk protein-specific or mammary-specific would be understood to refer to items which are found only in milk or mammary tissue. One of skill in the art appreciates that there are no codons which are used exclusively in milk or mammary tissue."

The claims have been amended to recite a "preferred mammary tissue-specific codon". The term "preferred codon" is defined at page 8, lines 28-29 of the present application as "a codon which is used more prevalently by the cell system." Thus, it is clear that the term "preferred mammary tissue-specific codon" refers to codons which are more prevalently used by mammary gland cells. Examples of such preferred mammary tissue-specific codons are set forth, for example, in Figure 3a. Thus, the amendment to the claims obviates this rejection.

Claims 4, 5, 8, and 9 are unclear because of the phrase "the known gene encoding is lowered by". Applicants have amended the claims to remove this language, thereby obviating this rejection.

In addition, Applicants have amended claims 8 and 9 to replace the language "the method of claim 5" with "the method of claim 6 or claim 7", thereby obviating the Examiner's rejection to these claims.

Claims 8, 10, and 11 have also been amended to provide antecedent basis for the term "the protein" and claims 10, 11, 22, 24 and 25 have been amended to replace the term "AT rich content" with "AT content". These amendments obviate the Examiner's rejection of these claims.

The Examiner also rejected claim 12 because "it recites malaria as a parasite" and claims 12 and 14-22 because they recite the term "specifically homologous." Claims 12 and 14-16 have been cancelled and claims 17-22 have been amended to depend from claims which do not contain the term "sufficiently homologous", thereby obviating this rejection.

For the reasons provided above, Applicants respectfully request that the Examiner withdraw this rejection.

Rejection of Claims 1-5, 11, 12, 14-16 and 22-24 Under 35 U.S.C. §102(b)

Claims 1-5, 11, 12, 14-16 and 22-24 are rejected under 35 U.S.C. §102(b) as being anticipated by random hexameric nucleic acids, product pd$(N)_6$ in the 1995 Pharmacia Biotech catalogue, page 277. According to the Examiner,

> Claims 1-5, 11, 12, 14-16, and 22-24 are product by process claims in which the process by which the product is produced carries no patentable weight. Th.e product is a nucleic acid which is a fragment of a modified known parasitic protein. The fragment may be of any length. The 1995 Pharmacia Biotech catalogue teaches random hexamer nucleic acids. The mixture of nucleic acids comprises all possible combinations of nucleic acid hexamers. Therefore, all two codon fragments of any nucleic acid are represented in this collection of random hexamers. Thus product pd$(N)_6$ anticipates the claims.

The claims have been amended to recite an isolated modified nucleic acid encoding a protein or fragment thereof. The 1995 Pharmacia Biotech catalogue only discloses a mixture of nucleic acid hexamers and not isolated nucleic acids. Thus, the product pd$(N)_6$ does not

anticipate the claimed invention. Therefore, Applicants respectfully request that the Examiner withdraw this rejection.

Rejection of Claims 1-12 and 14-26 Under 35 U.S.C. §103(a)

Claims 1-12, 14-19 and 22-26 are rejected under 35 U.S.C. §103(a) as being unpatentable over Dziegiel et al. (U.S. Patent Number 5,231,168), Holder et al. (1985) *Nature* 317(6034):270-273, Seed et al. (U.S. Patent Number 5,795,737), Akashi et al. (1994) *Blood* 83(11):3182-3187, Bosch et al. (U.S. Patent Number 5,736,131), and Wang et al. (1989) *J. Biol. Chem.* 264:21116-21121. According to the Examiner,

> Dziegiel teaches an expression vector comprising a nucleic acid encoding an antigen of Plasmodium falciparum. See abstract. The expression vector may be used in mammalian cells. See column 18, lines 54-65. The GC content of the nucleic acid is about 30%. See column 16, lines 40-43. Prior to use of the expression vector, the nucleic acid may be modified by silent nucleotide substitutions which favor the codon usage of the organism in which the nucleic acid will be expressed. See column 20, lines 66 to column 21, line 7; and column 21, lines 36-40. The nucleic acid may also be used as a vaccine, particularly as part of a virus. See column 25, lines 32-66. Dziegiel does not teach or suggest reducing the AT- content of the nucleic acid, the removal of the mRNA instability motifs or the introduction of codons found in milk- or mammary-specific proteins.
>
> Holder teaches the primary structure of the precursor to the three major surface antigens of Plasmodium falciparum merozoites. This sequence is 72.8% identical to SEQ ID NO:1 and 72% identical to SEQ ID NO:8, and can be considered specifically homologous to both SEQ ID NO:1 and SEQ ID NO:8. The coding region of this sequence comprises AUUUA motifs identical to those which have been found in 3'-untranslated regions of mRNAs. These sequence are known in the art, and identified in the specification, as mRNA destabilizing sequences. Holder also teaches that these antigens may be useful for immunization against P. falciparum. See abstract, and Fig. 2, page 272. Also see enclosed sequence alignments. Holder does not teach reducing the AT-content of the nucleic acid, the removal of mRNA instability motifs, or the introduction of codons found in milk- or mammary-specific proteins.
>
> Seed teaches that codon optimization may be used to increase expression of foreign genes in mammalian cells. See column 1, lines 8-10; and column 2 lines 7-11. Preferred codons are always those with the highest possible GC content. See lines 33-37, and Table 1, bringing columns 7 and 8. Synthetic genes comprising preferred codons can be expressed mammalian culture systems in

amounts in excess of 110% of the amount that the natural gene is expressed in the same systems. See column 2, lines 16-22.

Bosch teaches removal of mRNA instability motifs from nucleic acids which are to be expressed in heterologous hosts. Bosch also teaches codon optimization is advisable. See column 4, lines 12-21.

Akashi teaches that the function of AUUUA mRNA destabilization motifs is not restricted by their location within the mRNA. These motifs need not be located in the 3'-untranslated region of mRNAs, and are capable of destabilizing mRNAs even when located within the coding region. See abstract and Fig. 1.

Wang et al teaches a milk specific protein expressed in mammary tissue which comprises GC-rich codons. See Figure 2, column 1, page 21118, see particularly glycine codons at positions –11 and 18.

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the nucleotide sequences of Dziegiel or Holden by decreasing their AT-content and removing mRNA destabilization motifs. One would have been motivated to do so because Seed teaches that, of all the codons encoding a given amino acid, the preferred codon for expression in mammalian cells is the one with the highest GC-content. One would have been motivated to express the nucleic acid in mammalian cells because Dziegiel teaches the use of nucleic acid encoding a malarial antigen as a DNA vaccine. One would have been motivated to remove mRNA destabilizing motifs from the coding region of the nucleic acids because Akashi teaches that these sequences are active in the context of the coding region, and because Bosch suggests that mRNA-destabilizing motifs should be removed from sequences expressed in heterologous hosts.

It would have been obvious to one of ordinary skill in the art at the time of the invention to substitute codons found in milk- or mammary-specific proteins for those found in the nucleic acids of Dziegiel or Holden. One would have been motivated to do so because Seed teaches that GC-rich codons are preferred in mammalian cells. For example, glycine can be encoded by GGA, GGT, GGG, and GGC. The preferred codon is GGC, followed by GGG. See Table 1, column 7. The sequence of Wang comprises GGC glycine codons at positions –11 and 18. Thus, if one were to substitute a GC codon for a GGA codon as taught by Seed, one would be using a codon found in a milk- or mammary-specific protein.

Applicants respectfully traverse this rejection. The claims are directed to an isolated modified nucleic acid encoding a parasitic protein of a fragment thereof which can be expressed in a mammalian cell, e.g., a mammary epithelial cell, and methods of producing the modified

nucleic acid. The nucleic acid is modified by reducing the AT-content of the nucleic acid as it naturally occurs in the parasite by replacing at least one AT-containing codon in the naturally occurring nucleic acid with a preferred codon, e.g., a preferred mammary tissue-specific codon, encoding the same amino acid as the replaced codon and/or eliminating at least one mRNA instability motif present in the coding sequence of the nucleic acid as it naturally occurs in the parasite by replacing at least a portion of the mRNA instability motif with a preferred codon, e.g., a mammary-tissue specific codon, encoding the same amino acid as the replaced codon.

Dziegiel et al. disclose a parasitic protein, GLURP, generally, and a multitude of uses of DNA encoding GLURP, one of the many being codon optimized expression. As pointed out by the Examiner, Dziegiel et al. do not teach or suggest modifying the AT-content of GLURP or eliminating mRNA instability motifs of this protein. Moreover, this reference does not teach or suggest that mammary tissue-specific codon optimization can be used to increase expression in mammalian cells generally. Dziegiel et al. provide absolutely no motivation to consider codon optimization based on proteins expressed in mammary tissue. In fact, there is no discussion of codon optimization for any particular cell expression system. In addition, Dziegiel et al. do not generally teach or suggest expressing such a protein in a transgenic mammal, or in particular in mammary epithelial cells of a transgenic mammal.

Holder et al. is cited by the Examiner because of the level of sequence identity seen between sequence of the *Plasmodium falciparum* antigen disclosed in Holder and the sequences provided in SEQ ID Nos: 1 and 9. However, claims 12-16, which recite sequences sufficiently homologous to SEQ ID Nos: 1 and 9, have been cancelled. Since Holder adds nothing further to the Examiner's argument, this reference is no longer relevant to the patentability of the remaining claims.

Seed et al. add little. Seed et al. disclose codon optimization of naturally occurring eukaryotic and mammalian genes for expression in mammalian cells. The preferred codons cited in Seed et al. are based upon human genes that are highly expressed. There is no teaching or suggestion in Seed et al. that naturally occurring parasitic proteins would not be expressed in mammalian cells. Moreover, there is no teaching or suggestion in Seed et al. that preferred codons should be selected based upon mammary tissue codon usage. In fact, Seed et al. provide

no absolutely motivation to consider mammary tissue expression of proteins generally, and parasitic proteins in particular.

Akashi et al. studied the role of the AUUUA motif found in GM-CSF in RNA stability. Akashi et al. disclose that increased numbers of AUUUA cassettes may by a major determinate of the turnover of GM-CSF mRNA, that the AUUUA sequence when placed in an exon can still modulate stability of RNA and that AUUUA cassettes do not act alone in GM-CSF mRNA degradation. Akashi et al. did not study the effect of removing an AUUUA motif nor did they study the effect of removing an AUUUA sequence from the coding region of a nucleic acid sequence. Instead, Akashi et al. teach adding an AUUUA motif to determine the role AUUUA motifs play in RNA stability and report that AUUUA sequences alone do not play a major role in mRNA stabilization. See page 3186, column 2. Akashi et al. do not teach or suggest that naturally occurring parasitic proteins are not expressed by mammalian cells and that by removing a portion of an mRNA instability motif from coding region of the parasitic protein, these proteins can be expressed by mammalian cells. Moreover, there is absolutely no teaching or suggestion in Akashi et al. to replace a portion of the mRNA instability motif with a preferred mammary tissue-specific codon. Mammary tissue expression is not mentioned at all.

Bosch et al. disclose hybrid toxins derived from portions of *Bacillus thuringiensis* insecticidal crystal proteins. This reference generally discloses that DNA can be modified by removing instability motifs or using codon optimization to enhance expression in a heterologous organism. This general disclosure of modifying a bacterial protein, however, does not teach or suggest the claimed invention. In particular, there is no teaching or suggestion that a mammalian cell would not express naturally occurring parasitic proteins, or that by replacing codons in a naturally occurring parasitic protein with preferred mammary tissue-specific codons expression can be obtained in mammalian cells. In fact, there is no mention whatsoever of mammary tissue expression.

Thus, none of these references provide any suggestion to modify a nucleic acid encoding a parasitic protein to obtain expression in a mammalian cell since none of these references provide any indication that there would be a problem expressing naturally occurring parasitic proteins in mammalian cells. Moreover, none of these references provide any suggestion to use codon optimization based upon proteins expressed by the mammary gland to obtain expression

in mammalian cells generally. In fact, none of these references even mention mammary gland expression. Therefore, none of these references, alone or in combination, teach or suggest the claimed invention.

The final reference cited by the Examiner, Wang et al., does not make up for the deficiencies of the other references. Wang et al. disclose the expression of bovine α-lactalbumin in *E. coli* by expressing it as part of a fusion with the NH_3-terminal portion of cathepsin D. Wang et al. provide the nucleic acid sequence of bovine α-lactalbumin in a figure. Merely disclosing the sequence of a protein expressed by mammary tissue, however, does not teach or suggest determining preferred codons expressed by that protein. Moreover, it does not teach or suggest using mammary tissue-specific codons for codon optimization. It also does not teach or suggest that use of preferred mammary tissue-specific codons can lead to expression of a nucleic acid encoding a protein, especially a parasitic protein, in any mammalian cell.

Thus, the references cited by the Examiner do not teach or suggest the claimed invention. Moreover, none of the references cited by the Examiner, nor the Examiner's assertions made in hindsight, provide the motivation to combine the teachings of these references in order to arrive at the claimed invention.

Moreover, Applicants findings were unexpected. Applicants found that the naturally occurring nucleotide sequence encoding parasitic protein MSP-1 was not expressed at detectable levels in mammalian cells including mammary epithelial cells. Applicants surprisingly found that by modifying the nucleic acid sequence to replace codons in AT-containing regions and/or mRNA instability motifs with preferred mammary tissue specific codons, high levels of expression of the sequence occurred in mammalian cells. In fact, Applicants found that when this modified nucleic acid sequence was expressed in mammary epithelial cells, the parasitic protein could be expressed in the milk of a transgenic mammal at levels up to 1 to 2 mg/ml. Such results were not expected.

The Examiner further rejected claims 20 and 21 under 35 U.S.C. §103(a) as being unpatentable over Dziegiel et al., Holder et al., Seed et al., Akashi et al., Bosch et al. and Wang

et al. (1989) for the reasons discussed above, and further in view of Bleck et al. (U.S. Patent Number 5,530,177).

However, Bleck et al. merely disclose the nucleic acid sequence encoding bovine α-lactalbumin and methods of expressing bovine α-lactalbumin or other recombinant proteins under the control of a bovine α-lactalbumin control region. Thus, Bleck et al. do not make up for the deficiencies of the other references.

The Examiner also rejected claims 22-25 under 35 U.S.C. §103(a) as being unpatentable over Dziegiel et al., Holder et al., Seed et al., Akashi et al., Bosch et al. and Wang et al. (1989) for the reasons discussed above, and further in view of Robinson et al. (U.S. Patent Number 5,643,578).

However, Robinson et al. merely disclose genetic immunization against bacteria, viruses and parasites. This does not make up for the deficiencies of the other references.

For the reasons discussed above, Applicants respectfully request that the Examiner withdraw this rejection.

Conclusion

Applicants submit that all of the claims are now in condition for allowance, which action is requested. Filed herewith is a check in payment of the excess claims fees required by the above amendments and Petition for Automatic Extension with the required fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 10/12/00

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